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SOME PROBLEMS OF MODERN BIOCHEMISTRY IN THE USSR

By V. A. Engel'gardt

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SOME PROBLEMS OF MODERN BIOCHEMISTRY IN THE USSR

[Following is a translation of an article by V. A. Engel'gardt in the Russian-language periodical Uspekhi khimii (Progress in Chemistry), Moscow, Vol. XXVIII, No. 9, 1959, pages 1011-1035.]

Among all the divisions of modern chemistry, biochemistry is a field of particularly rapid and impetuous growth. In this respect it can be compared only with the chemistry of artificial polymers. Those fields of biology which have been invaded by chemistry are becoming more and more varied. The most important functions and characteristic specific features of living organisms--heredity, movement, activity of sense organs, energetics, the nature of diseases, the phenomena of immunity, and many others-all these fundamental manifestations of vital activity are becoming more and more the object of biochemical investigation with every passing day. A truly unbounded sphere of activity has been opened before modern biochemistry, and of all the most vitally important problems of biology, it would be difficult to find any which would not also be problems of modern biochemistry.

With the number and enormous variety of problems that face biochemical investigation today, certain dominant lines of investigation stand out in bold relief. These have created the present-day image of modern biochemical science and have determined the paths of its development in the near future. One can speak of central, decisive areas upon which research workers have concentrated their principal attention, and in which the achievements have been particularly great and the tempo of development most impetuous. In organic, and to some extent in inorganic, chemistry, the dominating place has been held in recent years by the study of synthetic polymers. In an analogous manner, the predominant attention in modern biochemistry has been concentrated on the study of two classes of natural high polymer substances: on the one hand, proteins; and on the other hand, amino acids. Of course each of these two dominant areas of modern biochemistry includes a large number of varied aspects which are now growing into large independent fields of knowledge, e.g., the aggregate of problems of enzymology, the problems of chemical genetics, etc. It is along these two lines that it is expedient to attempt to elucidate certain problems of modern biochemistry in this article. This restriction of the material examined is also justified by the fact that the great fields of research mentioned above are closely interwoven, so closely interwoven now that it is difficult to draw a boundary between them.

The role of the nucleic acids, or more precisely, one of them--desoxyribonucleic acid (DNA)--as a concrete chemical substance transmitting hereditary information to a number of generations has now become so generally known and so well substantiated that there is no need to dwell upon it in a generalized manner. The experiments showing that it is possible to use a DNA preparation obtained from one type of bacteria on microbes of another type and cause them to be transformed into the first type from which the DNA preparation had been made, were decisive in this connection. Since the change of properties brought about in this manner is inherited and maintained in a long series of subsequent generations, it is clear that the DNA acted here as a carrier of genetic information. In this connection, one should mention the very elegant experiments conducted by Straub in Budapest, which are as yet less well known [1]. They were concerned with the transmission by means of DNA preparations of the properties of sensitivity or resistance on the part of microbes to antibiotics. It was known that it was possible to obtain strains of bacteria that were resistant to penicillin by cultivating them in a medium containing first only very small, then growing quantities of this antibiotic. The cause for this resistance lay in the development of a special enzyme by the microbes, penicillinase, which destroyed the penicillin. Straub established that it was possible to obtain a DNA preparation from the resistant strain he had developed, and, by using this on a strain that was sensitive to penicillin, to transform it into a penicillin-resistant strain despite the fact that these bacteria had generally never come into contact with penicillin. Consequently, here too, DNA had acted as a carrier of inherited information and had brought about such a valuable property as resistance to a bactericidal substance. It is easy to imagine what sort of problems of paramount theoretical and practical significance originate in observations of this kind.

The structural model of DNA created by Crick and Watson [2] on the basis of physical studies specifies a double helix of two polynucleotide chains going in opposite directions, with the starting points of both chains so located that the adenine in one chain is always opposite thymine in the other chain while the guanine is opposite the cytosine. Both chains are held in place by hydrogen bonds which are broken at the time of cell division, when each single DNA chain that is formed acts like a matrix in forming a second complementary chain of the previous composition [3]. This is shown in Diagram 1 [see Figure Appendix].

This mechanism of self-reproduction of DNA during cell division, which was formulated speculatively, can be followed visually by means of an ordinary microscope. The object of study is first treated with thymine tagged with tritium. The thymine enters the DNA rapidly, both chains in equal measure. Upon division, each of the parental chains serves to form one of the two resulting daughter chromosomes. It was

possible to see by means of radioautographs that all the chromosomes of the first division (that is, in the first generation) contained a tagged atom, but it was now in only one of the chains. In the following (second) division it turned out that half of the chromosomes contained a tagged atom while the others did not have one [4]. This is explained by the diagram in Figure 1.

Thus, the mechanism of the duplication of DNA molecules which had been speculatively postulated became established.

The problem of genetic information acquires concrete form when written in a chemical code composed of a four-letter alphabet. The role of this code consists in the fact that it determines the particular structure of those proteins which are to be synthesized after cell division. These proteins form and compose a mass of daughter cells. It is postulated that each separate amino acid corresponds to a certain combination, that is, the composition and mutual arrangement of three pairs of nucleotide residues in the DNA chain. Here we have an example of the previously-mentioned close interweaving of the biochemistry of nucleic acids and the biochemistry of proteins. However, the actual forms of this connection are somewhat more complicated. The DNA serves as a matrix or a code of the first order. The synthesis of new cellular proteins is not carried out directly on it, according to the present-day ideas. The DNA serves as a matrix on which the ribonucleic acid molecule is synthesized, of course with a strictly-determined alternation of its purine and pyrimidine bases, whose location is determined by the location of the nucleotides of the primary matrix, that is, the DNA. Synthesized in the nucleus, on the DNA as on a matrix and receiving all details of its chemical code like a printed sheet from a stamp, the ribonucleic acid (RNA) then fulfills the role of the matrix of the second order. It is the RNA which participates as the direct pattern in the synthesis of protein which takes place in definite extranuclear sections of the cell, in its cytoplasmic formations. I shall pass on a bit later to the form of the participation of RNA in this most important process--the biological synthesis of proteins. First, I shall say a few words on the chemistry and the fate of DNA.

The splendid research done by Belozerskiy and his co-workers [5] has shown that not only the fine details of the structure of DNA (which we have not been able to detect as yet, but whose existence we assume on the basis of indirect considerations), but even the gross numerical relationship of the components which make up DNA, reveals characteristic differences in closely related types of microbes, or even in artificially obtained modifications of the microbes. In contrast to this, the composition of the RNA turned out to be practically the same at all times, thus obviously supplying all synthetic requirements of the cell with its components.

The biological task of DNA is to guard continuity in the change of generations. Any changes in its structure will lead to some changes in the progeny, to so-called mutations. It is natural, therefore, that

the DNA itself should retain as far as possible immutability of structure. This requirement is satisfied by the fact that the DNA in cells which are not dividing displays an extraordinary stability - an extraordinary metabolic inertness. The isotope method showed that in the period between divisions, DNA is almost completely free of cycles or rejuvenation in the cell. In this respect it differs sharply from all the other cell components which rejuvenate at a rapid rate - proteins, lipids, even ribonucleic acid. It follows from this that mutation occurs essentially as a result of imperfection of the process of self-reproduction or duplication of the DNA molecules in the process of division.

We know that if the stability and unalterability of DNA is a necessary condition for the continuity of inheritance, then mutations constitute a basic variability, and through this, evolution. Through natural or artificial selection they ensure the possibility of the improvement of organisms. We have the right to say that imperfection in the self-reproduction of DNA is the basis of the improvement of organisms.

In its physical and chemical aspects, on the molecular level or in "molecular biology," modern biology can find no more vital or urgent task than that of explaining the mechanisms of mutation, discovering their chemical nature, and through this finding ways to direct these mutations.

One large success has already been achieved along this path. We can direct mutations in a quantitative sense, that is, increasing their frequency by radiation or by chemical substances, the so-called mutagens. It should be noted that both these methods for quantitative influence on the process of mutation were discovered by Soviet scientists: Nadson [6] discovered the mutational effect of X rays on yeasts. Rappoport [7] discovered the mutagenic effect of chemical substances and found some compounds which had a particularly energetic mutagenic action. No matter how effective radiation may be as a mutagenic factor, it has one exceedingly important shortcoming: according to theoretical considerations one must expect that the character of the mutations caused by radiation will be completely random and that one cannot count on any selectivity at all. In contrast to this, we have a right to expect some degree of selectivity from chemical mutagenic factors; thus they should be of special interest. Experimental evidence already exists in favor of the selectivity of the mutagenic action of chemical substances. When the action of several mutagens was compared, namely: 5-bromouracil, proflavine, and 2-aminopurine (Diagram 2), it turned out that each of them caused the appearance, as Benzer stated [8], of "hot zones," that is, zones in different places in the linear structure of the same gene, where mutation could occur with particular ease. Even though this is still a most incomplete result, it is important in that it provides evidence of the theoretical possibility of selective, localized action by this or that mutagen.

In the case of the example given here, it must be emphasized that the mutagens used had the character of the factors which have been designated as "antimetabolites." These are compounds which have structures similar to the normal components of nucleic acids but still are not identical to them and differ from them in a definite manner. One is compelled to believe that their action in mutation is caused by their being substituted for certain normal components in the process of the self-reproduction of genes, thus giving rise, so to speak, to their own type of chemical mutilations in the newly synthesized nucleotide DNA chain. Consequently, we have here an exceedingly interesting example of the application of the principle of antibiotics on the level of genetic experiments. Undoubtedly this can offer prospects enticing to the highest degree.

The problem of the chemical formation of mutations is indivisibly connected with the problem of the methods for the synthesis of polynucleotide chains of both nucleic acids. Important progress has been achieved in these methods in recent years. The most splendid achievements of biochemistry in the last 5 years must certainly include the outstanding successes achieved in the fermentative synthesis and the contributions of Ochoa, Grunberg-Manago, and a number of other coworkers [9] on the one hand, and Kornberg [10] with his group on the other. A number of fundamental facts have been established, as for example, the necessity for the presence of some "primer," that is, preformed nucleic acid. It was established that in some cases synthesis proceeds along the path of the splitting of orthophosphate; and in others, the separation of pyrophosphoric acid from the corresponding phosphorylated forms of nucleotides. The possibility of building up a polynucleotide chain on two of its ends was demonstrated with the hydroxyl group in the third hydrogen pentose, or from the end polyphosphate group as shown in Diagram 3.

The problem of the nature of the "priming" action and, along with it, the problem of the factors which control the ordered alternation of the nucleotides in the chain in accordance with a definite predetermined plan, constitute a larger problem of great significance.

Whether the "primer" serves as a matrix, whether it acts only as an acceptor of nucleotide residues for building up the chain, or participates in the process in still some other manner, remains unknown. In one way or another, from investigations of this sort we have the right to expect to discover the nature of the mechanisms which control the alternation of the links in the structure of the nucleic acids, and to then proceed from that point to learning how derangements can take place in the formerly established order, that is, how mutations take place. There is no doubt that this is one of the most important problems of modern chemical biology.

If we wished to summarize what has been said concerning present-day trends in the study of nucleic acids and to note the chief problems which stand before research workers in this field, the most urgent of

these problems would undoubtedly be the problem of establishing the chemical structure of the nucleic acids. The Watson-Crick diagram gives an idea of the general plan of the structure of the nucleic acids rather than the physical aspect of this structure. Priority should be given the task of decoding the sequence of the location of the nucleotide links in the enormous nucleic acid chain. This is a task of no small difficulty and, which is not strange, the chief cause of this difficulty may be in the comparatively great simplicity of the composition of the nucleic acids. It is clear from the brilliantly successful experiment which clarified the structure of proteins that the way to decode the sequence of the links in the long linear polymer consists of fragmentation, breaking the long chain into a number of shorter segments accessible to chemical analysis. Comparing the composition of the fragments obtained by painstaking selection, a process reminding one of the assembly of a jig-saw puzzle in which a complicated picture is broken down into a large number of pieces, will ultimately make it possible to establish the sequence of the location of the links in the original chain. In the case of proteins this difficult task is lightened by the fact that we have here a variegated assortment of primary links--about 20 amino acids. Since different enzymes break only the linkages between definite pairs of adjacent amino acids, it is possible to divide the polypeptide chain into different segments and to obtain fragments of different composition. On the other hand, the variety of the amino acids and their comparatively large number provide a considerable number of support points when reconstructing the "jig-saw puzzle," that is, establishing the original sequence of amino acids, since one can exclude one of the assumed combinations after another and, on the other hand, find possible combinations. In the case of the nucleic acids, we have only four elementary components, four types of bases. We do not have at our disposal as yet preparations of those specific enzymes which would break only certain types of linkages. Here it is almost impossible to expect such specificity, as the broken linkages are not found between specific components of the nucleotides, not between their purine or pyrimidine bases, but between pentose residues which are identical in all links. Apparently it is necessary to wait for the development of some fundamentally new methods of analysis in order to solve this first-priority problem in the field of the chemistry of the nucleic acids.

It was natural to devote special attention to examining the problems connected with the chemistry of DNA and the resulting prospects for studying problems of genetics by physical and chemical means. This was justified by the vital importance, both theoretical and practical, of this entire sphere of problems of modern biochemistry.

I shall now pass on to an immediately adjacent field - that of the mechanisms of the synthesis of proteins - a synthesis which, as pointed out previously, is controlled by and progresses with the close participation of RNA.

The process of synthesis of the protein molecule has not been divided into a number of isolated, sharply bounded stages [11]. Originally, three of these stages were separated. The first stage was the activation of the amino acids to be joined together by the formation of peptide linkages. The second stage is the ordered combination of the activated amino acids by closure of the peptide linkages; this is now called "sequentialization" (it is difficult to find a single word which conveys the idea of this term), that is, arrangement in a given order. The third stage is the acquisition of a definite arrangement in three-dimensional space that is specific for a given protein by the linear molecule of the polypeptide chain - the acquisition of a three-dimensional structure.

Of these three stages, we have concrete chemical ideas of only one - that of the activation of amino acids. As for the other two stages, which are actually particularly important, as they determine the entire specifics, that is to say, the individual chemical physiognomy of each individual protein, the task of clarifying the mechanisms and patterns which direct them still remain a matter for future research.

The carboxyl group and the amino group of the amino acids are not reactive enough to link directly with one another to form a peptide linkage. In his classical experiments on the synthesis of the first peptides, Fisher increased the chemical mobility of the carboxyl group by using the anhydride in place of the acid in the form of the acid chloride. By proceeding in this manner, the great chemist followed in the footsteps of nature. It is in this way, the use of the anhydride of the amino acid, that its activation is accomplished in the process of biological synthesis. The chlorine used by the chemist is not very suitable for this purpose; it is difficult to imagine that amino acids are activated in the cell by phosphorus pentachloride, as the organic chemist does it. Nature has chosen a way which is theoretically similar in chemical respects, but incomparably more gentle still, due to the participation of enzymes, a very effective method. Instead of chlorine, a nucleotide (adenylic acid) is included in the anhydride linkage. In chemical respects the process of activation is the result of splitting the pyrophosphoric groups from the adenosinetriphosphoric acid and replacing them by aminoacyl; that is, this reaction can be regarded as an inversion of the so-called pyrophosphorolysis (Figure 2).

The adenyl anhydride of the amino acid is formed; it is the "activated form" of the amino acid. This activation reaction takes place in the liquid, structureless part of the cytoplasm under the effect of special enzymes. As far as one can judge at present, every individual amino acid requires its special enzyme for activation. Many of them have already been separated into their individual form. As in the majority of anhydrides, the linkage in the aminoacyl adenylate has an increased supply of energy - this is the "rich energy," or the macroenergic linkage.

The second state which follows the activation of the amino acid is of great interest. Here RNA is included in the process where it is a definite, unique fraction. This is the so-called "soluble" RNA of the cytoplasm which is not included in the subcellular structural elements of the ctyoplasm - the microsomes, the mitochondria, etc. This soluble RNA fulfills the unique function of transporting the activated amino acid from the activating enzyme to the place where the peptide linkage is actually formed: the activated amino acid residue is transferred from its linkage with the adenyl acid to a linkage with a molecule of ribonucleic acid. This transaminoacylation, as we might call it, is ascribed to the same activating enzyme that caused the primary formation of the aminoacyl anhydride, that is, the activation of the amino acid. The "soluble" RNA is distinguished by a comparatively low molecular weight, on the order of 10,000-40,000; that is, it contains 20-80 nucleotide residues. In order that the amino acid may be linked with the RNA, its end portion must contain a certain combination of nucleotide residues, namely, the sequence of adenyl and cytosine nucleotides. It is not clear yet whether the components of this group serve at the place where the amino acid has been transported or whether, on the other hand, it is held elsewhere in another part of the polynucleotide chain while the end group is essential only as a contact point for the enzyme. There are indications that the contact of the amino acid takes place in the hydroxyl group in the hydrogen pentose which occupies position 2 or 3.

The role of the so-called "soluble" RNA is defined to be the function of an "adapter": its task is to transport the activated amino acid with its inherent supply of chemical energy to the structural elements of the cytoplasm (microsomes and mitochondria) where the arrangement of the amino acids is actually carried out in the required order and where the synthesis of the peptide linkage takes place, that is, in the actual construction of the protein molecule.

In the initial stage, the participation of one polyphosphorylated nucleotide, adenosinetriphosphoric acid, is required for activation of the amino acid. In the final stages the participation of another purine mononucleotide, the guanine, also in the triphosphate form (in the form of guanosinetriphosphoric acid), has been found to be necessary. Although the chemical concept of participation is clear for ATP, nothing definite can be said as yet concerning the form of participation of GTP. It turns out in general that the more closely we approach the final stages of the synthesis of protein, the more scanty our knowledge of certain chemical mechanisms which occur there. Therefore it is necessary to limit ourselves to presenting the general diagram which sums up what we have stated here (Figure 3).

There is no need to emphasize how large the number of questions is that face the research worker in the field just now discussed - the problem of the biosynthesis of protein. The number, the variety, and the vital importance of all the ramifications which originate in this

central problem are perfectly clear, and it would be superfluous to enumerate them here. Instead, it would be expedient to go directly to a discussion of certain aspects of the study of this product, a subject to which we have been led by the foregoing elucidation, that is, to go on to problems connected with the study of the chemical structure of protein.

The chief attention has been concentrated here at present on studying the biologically active proteins, chiefly two categories: the enzymes and the protein or polypeptide hormones, also (more on a physical plane) the biologically active proteins - pigments, chromoproteids, in particular hemoglobin and myoglobin (the coloring substance in muscles). This is not at all surprising, since in this case the study of purely chemical problems is indivisibly connected with the solution of the most important problem - that of the connection of biological activity with certain details of chemical structure.

The present era of the structural chemistry of protein was founded by Sanger [12], who discovered the structure of the entire amino acid skeleton of insulin. In the course of this classical research he developed a complex of methods which are the basis for the present work on the structural chemistry of proteins. There is scarcely any necessity for enumerating or presenting these methods; it is sufficient to state that two main principles are used: fragmentation of the original protein by different proteolytic enzymes (trypsin, chymotrypsin, subtilysine, and others) into more or less large fractions, and determination of the structure of these fractions by establishing the end groups, the so-called N- and C- end groups, that is, those containing the amino group or the carboxyl group. The chain segments were shortened by splitting off the end residues by chemical means (the phenyl isothiocyanate method with the formation of hydantoins for N-end groups and hydrazinolysis for C-end groups), or by an enzyme method, by the action of amino- or carboxypeptidases. After establishing the order of arrangement of the amino acids in each individual fragment, comparisons of the formulas of the individual fragments which had been obtained in this manner permitted determining the sequence of arrangement of amino acid residues in the entire original protein molecule after much painstaking and laborious work.

Although the solution of the protein "jig-saw puzzle," that is, establishing the sequential order of amino acids in a protein molecule, continues to be a laborious task, determining the general amino acid composition of any protein, not too long ago a time-consuming and laborious project, has now become a simple technical operation. An automatic device has been built, of not too complicated design, which has a volume of about half that of an ordinary chemistry table [3]. The protein hydrolysate is placed in the device, and in 24 hours the automatic device itself makes a complete analysis without any intervention by the experimenter, and gives the quantitative results in the form of a graph inscribed on a tape, from which it is easy to find the absolute quantitative content of individual amino acids.

TABLE

STRUCTURE OF THE INSULIN MOLECULE

	B	A
1	Phenylalanine	Glycine
2	Valine	Isoleucine
3	Asparagine	Valine
4	Glutamic acid	Glutamic acid
5	Histidine	Glutamic acid
6	Leucine	Cysteine
7	Cysteine ------- S ------- S -------	Cystein
8	Glycine	Alanine
9	Serine	Serine
10	Histidine	Valine
11	Leucine	Cysteine
12	Valine	Serine
13	Glutamic acid	Leucine
14	Alanine	Tyrosine
15	Leucine	Glutamic acid
16	Tyrosine	Leucine
17	Leucine	Glutamic acid
18	Valine	Asparagine
19	Cysteine	Tyrosine
20	Glycine ---- S ------- S -------	Cysteine
21	Glutamic acid	Asparagine

B TABLE (CONTINUED) A

22 Arginine

23 Glycine

24 Phenylalanine

25 Phenylalanine

26 Tyrosine

27 Threonine

28 Proline

29 Lysine

30 Alanine

We have presented a table of the structure of the insulin molecule. The molecule consists of two polypeptide chains: one (Chain A) consists of 21 amino acid residues; the second (Chain B), of 30 residues. The chains are connected by two disulfide bridges: one between the cysteine residues in position 7 in both chains, the second between the cysteines in position 20 in the A chain and position 19 in the B chain. In addition, there is an intrachain disulfide bridge which forms a 20-atom cycle.

Two hormones of polypeptide nature produced in the hypophysis cerebri have been the object of thorough chemical study. They are vasopressin and oxytocin, which act on the muscles of the uterus and blood pressure. They turned out to be peptides which contain nine amino acid residues and 20 element cycles formed by disulfide bridges (refer to Diagram 4). Decoding their structure was completed by successful synthesis of both hormones [14]. This first synthesis of natural biologically active peptides represents one of the brilliant pages of contemporary biochemistry and organic chemistry.

When we examine the formulas of vasopressin and oxytocin, our attention is directed first of all to the fact that two hormones with entirely different actions have structures that are exceedingly similar - with just the differences, in the first place, that the aliphatic amino acid isoleucin is replaced by an aromatic one, phenylalanine, while in the other part of the chain the neutral amino acid, leucine, is replaced by the basic amino acid arginine. Such is the difference between the two different hormones. It turned out that no matter what species of animal one used as a source of oxytocin, it always had exactly the same structure. The situation was otherwise in

the case of vasopressin. As may be seen from comparing the last two lines in Diagram 4, the hormones from cattle and pigs differed in one link of the peptide chain: in both cases the amino acids are basic, but in cattle vasopressin it is arginine while it is lysine in pigs. This difference has no effect whatever on the biological properties.

This example introduces us to a field which is of great interest - concerning differences in species and structural aspects of biologically active substances, also all their most important categories which belong to the general class of compounds of peptide structure: peptides and protein hormones, enzymes, the respiratory pigments - hemoglobin and cytochrome. One may state that the progress made in protein chemistry of recent years has opened a new page in chemical biology and has made it possible to speak of comparative anatomy on the molecular level. Although comparative anatomy, which had established the kinship and difference of species and other systematic categories, had operated by comparing organs and other large parts of the body, their forms, structures, etc., up to this time, it has become possible now to speak of the anatomy of molecules. This is one of the new and recent large problems of modern biochemistry and it merits attention.

At present, the structure of a considerable number of polypeptide-like hormones has been established in the sense of the order of alternation of amino acids. Except for insulin, these are hormones from the hypophysis cerebri. In a number of cases they are hormones of "higher order": although they themselves are products of internal secretion, they control in turn the activities of different endocrine glands - the thyroid, adrenal, and sexual glands. The hormones of the hypophysis include the above-mentioned two low-molecular hormones, vasopressin and oxytocin, then the unique hormone which controls the activities of the pigment cells of the skin in amphibians, the so-called melanotropic hormone; the hormones of growth, the lactogenic hormone which controls the process of the formation of milk in the mammary glands, etc.

Complete decoding of the alternation of amino acids in their molecules has been achieved for some of them. This was achieved in the case of the exceedingly important hormone ACTH (adrenocorticotropic hormone) with its 39 amino acid residues, and also in the case of the melanotropic hormone which contains 18 residues.

Two similar, very important patterns were discovered in almost all cases [5]. On the one hand it was almost a general rule that similar functions were fulfilled in all representatives of the animal world studied by compounds which were practically identical in respect to the general structural plan. At the same time, however, in a number of cases there were definite, as a rule very small, differences in the sense that in certain places the molecules of some amino acid residue would be replaced by another. We saw this also in the example of vasopressins. To illustrate this point further we can present the following comparisons.

Differences in species were also discovered in insulin. Here, in this large molecule containing 51 amino acids in its two chains, the possibilities for all sorts of changes would seem to be very large. However, it was actually found that in some cases the hormone of species far apart in the systematic series of species - pigs and whales - were completely identical. Differences were observed in other species, but it is noteworthy that all of them were concentrated in one closely limited section of the molecules, namely, in positions 8, 9, and 10 of chain A, within the bounds of the intramolecular cycle closed by the disulfide bridge (Diagram 5).

Sheep insulin differed from cattle insulin by the presence of serine in place of glycine. Horse insulin differed from the sheep hormone by two amino acids: alanine in place of threonine and valine in place of isoleucine. Finally, insulin from pigs and whales which, as has been stated previously, was identical, differed from the horse hormone by one amino acid and in this case serine replaced glycine. Consequently, position 8 might contain either alanine or threonine; position 9 might contain either serine or glycine; and in position 10, either valine or isoleucine. With all these changes, the biological activity remained constant. It may be concluded from this that the section inside the disulfide loop in the A chain is not directly responsible for the biological effect; it does not serve, as it is now acceptable to say, as the "core" or active center which determines this or that biological function of the protein, in this case its hormone function.

Of great interest is the fact that two biologically active proteins with wholly different functions, for example, two entirely different hormones which possess very different general structures, sometimes display quite large sections of their structures which have identical amino acid arrangements. Two hormones from the hypophysis can serve as an example of this: the adrenocorticotropic hormone on the one hand and the melanotropic (which stimulates the pigment cells) on the other. The adrenocorticotropic hormone (ACTH) contains 39 amino acid residues, and the melanotropic hormone is of considerably simpler structure - only 18 residues. At the same time, their molecules contain parts which show an exceedingly high similarity in the sequence of the amino acid residues and are entirely identical over a considerable length (seven residues) (Diagram 6).

Loops are identical in both hormones. We see identical heptapeptides and a unique exchange of places by adjoining serine and lysine, then again identical tyrosine and proline residues, such that if it were not for the above-mentioned interchange of the two amino acids, the identical sections would be extended to a sequence of 11 amino acids, that is, more than half of the molecule for one hormone and a fourth of the total molecular structure for the other.

We see from the examples cited here that the problem of the biological action of the hormones, these mighty regulators of metabolism in the organism, has now been shifted to a large extent to the plane of the chemical structures of molecules.

Another no less important and considerably larger group of substances of prime importance where the problem of the biological, specific action is now becoming an ever-growing subject of study of molecular structures, are the enzymes.

After the decoding of the structure of insulin, the clarification of the structure of the enzyme ribonuclease, which is now almost completed, should be considered as an equally great event [16]. In contrast to insulin with its two chains, it has a single polypeptide chain of 129 amino acid residues. Eight cysteine residues form four disulfide bridges. The location of the cysteines in the chain and the clarification of which pairs formed disulfide linkages between themselves, made it possible to construct the hypothetical spatial structure which the peptide chain should have in order to ensure the possibility of closing the above-mentioned sulfide linkages (Figure 4).

Thus it was possible for the first time not only to establish the composition of the enzyme, not only to clarify the sequence of the amino acid residues in its peptide chain, but also to sketch probable contours for the general configuration of the enzyme. In the light of the information obtained, it also turned out to be possible to clarify certain questions in regard to connecting the structure with enzyme activity. We shall return to this a bit later.

The structure of ribonuclease presented here was constructed by research workers on the basis of purely chemical studies. This is a structure represented in a single plane. By utilizing other approaches of purely physical character, it has become possible to attempt to give a three-dimensional structure of the protein molecule. Use of new methods of X ray structural analysis has permitted the construction of a spatial model of one of the biologically active proteins - myoglobin [4]. This is a close analog of hemoglobin contained in muscles.

In returning to the field of the chemical study of biologically active proteins, it is necessary to emphasize the results obtained from studying certain details of the structure of another protein which belongs to the same group as myoglobin, that is, to the hemin proteids [17]. This is cytochrome, which is an important and universally prevalent catalist in cellular respiration. The details of the structure of its peptide chain are still far from being as thoroughly clarified as they are in ribonuclease, but to make up for this certain features of the same "comparative anatomy" sort on the molecular level, of which we spoke in connection with hormones, stand out.

In cytochrome we have a hemin structure, similar to that contained in hemoglobin, which has thioether linkages with the protein base. The hemin itself is not catalytically active. It acquires its activity as a result of being connected to a molecule of a specific

protein. It is natural to expect that the section of the protein molecule to which the hemin is connected will be of decisive importance in activation. It turned out to be possible to separate that part of the chain with which the hemin was linked from the general peptide chain of the cytochrome protein by means of careful hydrolysis which did not destroy the thioether linkages. The so-called hemopeptides were obtained with 9 links and 12 links and the sequence of the amino acids in them was established. It was found that all the corresponding sections were identical for three mammals under study - cattle, horses, and pigs (Diagram 7). In chickens, alanin was replaced by serine in the section between cysteine residues. In silkworms the difference involved the amino acid which stood outside the thioether linkages in immediate proximity to them; here the lysine was replaced by arginine. Finally, in passing from the animal world to lower organisms, that is, to yeasts, the location of arginine was found to be the same as in birds, but both the amino acids between the cysteine residues were different: here glutamic acid and leucine were found, but the further sequence of amino acids (in the direction of the C-end section) was identical with all the others. Thus we see an exceedingly strong continuity in the general structural plan of the responsible part of the protein contained in the cytochrome throughout the world of living things - the distance between the cysteine residues and the presence of an immediately adjacent histidine residue, to which an important role has been ascribed in the formation of coordinate linkages with the iron of the hemin nucleus.

The question of the connections and dependent relationships between the chemical structures and the biological functions of biologically active substances is one of the fundamental problems of biochemistry. Let us touch upon two aspects of this problem which appear to me to be of particular interest. In the first place, this is the problem of the mechanism of activation of those catalytically inert forms, the so-called zymogens, the form in which a number of enzymes are produced by cells. In particular, this concerns the proteolytic enzymes. The very fact that the digestive enzymes are produced in an inert form and that they are subsequently activated was once the object of careful study by I. P. Pavlov who worked with Shepoval'nikov to discover the existence of a special factor in intestinal juice, the so-called enterokinase [18] which activated the proteinase of the pancreas gland, especially trypsinogen. Later this process of transforming trypsinogen into active trypsin (this can also take place autocatalytically under the action of preformed or added trypsin) acquired a concrete chemical interpretation.

We are still far from any complete decoding of the peptide structure of trypsinogen or trypsin. However, certain features of their structure have been established [19]. The schematic structure of trypsinogen, as it may be outlined on the basis of available data, is given in Figure 5.

The alternation of amino acids in the nine links of the N-end section of the molecule has been established; the presence of intramolecular disulfide bridges which form loops has been established. There are strong indications that an important role is played in carrying out the catalytic function by one of the serine residues on the one hand and by a histidine residue on the other hand.

It was found that the process of activation, the transformation of trypsinogen into active trypsin, consists (in chemical respects) in splitting a hexapeptide segment from the N-end section - valyl-tetraasparagyl-lysine. The results of physical research, especially changes in optical rotation, provide evidence that the degree of spiralization of the peptide chain is increased as a result of the splitting off of this hexapeptide, and it is believed that the serine and histidine residues contained in the "active center" which were previously separated spatially have thus been brought into proximity. The breaking of the lycyl-isoleucine linkage with the splitting off of the hexapeptide removes the previously-existing spatial obstacle caused by the presence of hydrogen bonds and makes possible the occurrence of the spatial configuration which possesses catalytic activity.

Another pancreatic enzyme, chymotrypsin, is also produced in the gland in an inactive form, chymotrypsinogen, which is activated by trypsin. Here the basic process of activation consists in breaking a single peptide linkage, but this break takes place in some closed peptide loop and is not accompanied by the splitting off of a free peptide as in the preceding case. Following activation, however, one can trace a secondary process which consists of a dipeptide group from one of the segments produced by the previous break. This breaking off of the dipeptide is not accompanied by any change in enzyme activity, which indicates the possibility of measurable changes in the protein structure without any destruction of its basic biological properties. In this case, we are dealing with the breaking off of a very small part of the molecule, on the order of 1 percent. However, cases are not rare in which it is possible to wreak incomparably more damage to the peptide structure of a biologically active protein without any noticeable effect on its specific function. It may be that this is one of the most unexpected discoveries in the field of enzyme chemistry in recent years.

Thus it was found in experiments with ribonuclease [16] that fairly thorough processing with aminopeptidase, progressively shortening the peptide chain from the N-end section, is without affect on the enzyme activity. By using a proteolytic enzyme of bacterial origin, subtilysine, it turned out to be possible to split off a very large section of this region of the peptide chain, approximately within the limits of the first 20 amino acids without damaging the activity. It was clear from this that the nitrogen end of the peptide skeleton of the ribonuclease molecule was not needed at all for its

activity. It is obvious that the latter was connected with some part of the molecule located closer to the carboxyl end. However, it was possible to shorten the carboxyl end, too; for example, carboxypeptidase could split off a minimum of three of the C-end amino acid residues without destroying enzyme activity. Thus it was found that as many as 25 amino acids of the total of 129, that is, about 20 percent of the components of the enzyme molecule, were not vital and were, so to speak, surplus in respect to enzyme activity. According to a verbal statement by the author of this research, K. Anfinzen, it is apparently possible to wreak still more thorough destruction on the structure of the ribonuclease molecule, namely, breaking the peptide linkages in sections closer to the center. As long as the disulfide linkages which fix the secondary spatial structure are preserved, the catalytic activity will also be preserved.

Very similar results were obtained by studying the enzyme enolase [20]. Here the absolute number of amino acid residues which could be removed without damage was 4 times as large, reaching 100 amino acid residues. In view, however, of the considerably greater molecular weight of enolase, we have about the same percentage relationship as we had in the case of ribonuclease - about 17 percent of the total components of the enzyme could be removed without destroying activity.

The facts discovered in studies of the vegetable enzyme papain were undoubtedly the most astounding [21]. Its molecule is built up of 180 amino acid residues; it was found that up to 120 residues could be removed, that is, over 70 percent, yet 99 percent of the initial enzyme activity would be preserved!

The aggregate of observations which have been made leads to the undoubted conclusion that apparently, as a rule, the catalytic activity of the enzyme molecule (and one may add, similar observations are to a certain extent available in respect to certain peptide hormones) is not determined by the entire chemical structure and the configuration of the molecule as a whole, but is concentrated in some restricted section of the molecule. This section which is responsible for the biological properties of the protein is now called the "core" or "active center."

The idea which has become firmly fixed in biochemical thinking of the existence of some "core" in a molecule of biologically active protein, in which the corresponding activity (enzyme, hormone, or immunological) is concentrated, and of sections which play no part in this activity, is an idea which has posed two questions of essentially almost equal importance. On the one hand, it is naturally extremely important to clarify the structure of the active section or "core" in all its details, thereby searching for a way to learn the very mechanism which carries out the corresponding specific function. However, the question of the role of the "unneeded" parts of the molecule which can be removed without destroying the fundamental biological action of a

given protein is also of interest. It is not too easy to reconcile oneself to the thought that with such perfection in selecting the most effective physiological mechanisms and chemical structures, nature could display unusual wastefulness here and, for example, burden the "core" of papain with a mass of unnecessary ballast material 3 times its own mass. It is believed that this matter is not so simple and that it would pay to continue the search more persistently for those functions and effects which are carried out by these parts of molecules of biologically active substances which seem at first glance to be unnecessary. This is one of those problems which is wholly unsolved as yet.

Biochemistry is extending its sphere to a number of diseases, making it possible to interpret their nature in terms of the chemical structure of the molecules, or in terms of the interaction of chemical substances, or finally, in terms of chemical reactions caused by enzymes. In his own times Virchow created the concept of cellular pathology - a concept which searched for an interpretation of morbid disorders on the cellular and tissue level. Now it has become possible to speak of "molecular" pathology, not of cellular pathology. Pauling has introduced the term "molecular disease," and had in mind changes in the structure of a certain type of molecule, some "malformation" of molecules which in turn would lead to some disorders in the organism. The changes would be the cause of the disorders. This term "molecular diseases" has won good standing rapidly, it is finding more and more wide usage, and the number of "molecular diseases" is growing constantly.

One of the blood diseases, the so-called sickle-cell anemia, is the best example of "molecular disease" which gave rise to the concept expressed in this term. This disease is marked by the fact that the erythrocytes acquire a sickle-like or half-moon form. This is the external indication; for the patient it is important that it is accompanied by a number of other serious symptoms. The disease is congenital, hereditary, and under certain conditions it can even be deadly.

The formation of sickle-like erythrocytes is caused by decreased solubility of hemoglobin and it crystallizes inside the erythrocyte in these patients. The hemoglobin crystals aggregate, forming a so-called tactoid structure, and give the blood corpuscles the sickle-like form. Research has shown that hemoglobin from the patients (it is called hemoglobin S from the word sickle) has an isoelectric point that is different from the normal - a different mobility in an electrical field. The sharp difference in solubility (appearing in the reduced form) has already been mentioned. Partial splitting of hemoglobins, normal and sickle-cell, has been accomplished by trypsin, and this has led to forming about 30 peptide fragments. Comparison of these peptides by combined paper chromatography and cataphoresis showed that the difference is in one of these fragments. This principle of comparing two-dimensional chromatograms or electrophoregrams of peptides in order to distinguish proteins has now become most widespread. It is called the

"dactylogram" method (in English "fingerprint"), that is, it resembles the comparison of fingerprints [22]. Let us present as an example such a "chemical dactylogram" of the above peptide mixtures of the two hemoglobins (Figure 6). The peptide which is different from the normal is crosshatched. The structure of this peptide was determined and compared with the corresponding normal peptide [23]. This was a nine-element nonapeptide. Its structure is given in Diagram 8 for three types of hemoglobin.

We see that in all cases the difference involves the same link. In normal hemoglobin we find a glutamic acid residue at this point. In sickle-cell anemia the glutamic acid is replaced by neutral valine, thus this hemoglobin has an excess positive charge as compared with the normal. In hemoglobin C a lysine residue replaces the glutamic acid and the molecule has a still more basic character.

It is most noteworthy that the given differences in the amino acid composition turned out to be the only ones throughout the entire hemoglobin molecule. All the other peptides are absolutely identical. The hemoglobin molecule contains approximately 300 amino acid residues and "molecular disease" is due to the fact that just one of the 300 amino acids has been changed. As may be seen in Diagram 8, this very same amino acid is changed in another type of hemoglobin - in hemoglobin C.

Sickle-cell anemia is a congenital, hereditary disease. Study of the laws of its inheritance showed that it is inherited in strict accordance with Mendel's laws. We have here a typical case of Mendelian inheritance which can be reduced to a change in the structure of just one link in one definite protein molecule. We are not able as yet to decode and write down the structure of a gene, but the research cited here will permit describing chemically the amino acid sequence in the peptide chain whose synthesis is controlled by the corresponding gene.

It is accepted that the localization of each individual amino acid residue in a peptide chain of this or that protein is determined by the specific arrangement of three nucleotide residues in the DNA molecule, that is, in the genetic code of a chromosome. It follows from this that inasmuch as we are dealing with changes in the character of one amino acid residue in this case, this very small difference in the peptide chain reflects just as small a difference in the polynucleotide DNA chain, perhaps involving just a single link in that chain.

Thus the given case may serve as a specially vivid example of ultimately deep penetration of purely chemical research into the field of the greatest biological problems: on the one hand the problems of inheritance, and on the other hand the problem of pathogenesis - the primary cause of disease. This is an example, in the first place, of genuine "genetic chemistry" and in the second place, of genuine "molecular disease." Here a hemoglobin molecule is stricken with disease!

Inasmuch as we have been discussing the chemical interpretation of disease, I shall mention, of necessity very briefly, still several examples in which the same depth of penetration into the molecular level has not been achieved as yet, but nevertheless the chemical interpretation has already brought us far toward understanding the nature of disease. In turn, knowledge of the nature of disease will give us a basis for rational searches for ways to intervene in and to control the corresponding disorders.

Congenital jaundice of children is one case. It was found that this disease was caused by either lack or decreased activity of one enzyme, namely, the specific glucuronidase, which causes formation of a biliary pigment, bilirubin, from glucuronic acid. Only in this complex is bilirubin capable of undergoing normal transformations, transportation processes, penetration into the tissues, etc.

As in the case of sickle-cell anemia, this disease is congenital and hereditary. There are good grounds for assuming that here, as in the anemia, the gene which controls the production of the corresponding protein has been damaged - in this case the enzyme glucuronidase. It is a matter for the future to determine what the advancing disorder is: has production of the corresponding enzyme protein ceased entirely? or is it being produced, as in the case of sickle-cell anemia, in the form of a protein which differs only a little in structure from the normal? - this difference involving the responsible section of the molecule and being accompanied by a loss of the corresponding specific catalytic capacity so that the "misshapen" protein is no longer an enzyme. If this turns out to be the case, then we should have here a molecular disease - a damaged or misshapen enzyme molecule.

Another disease which also merits mention is the so-called Wilson's disease, or hepatolenticular degeneration. This is a hereditary disease which affects the nervous system and is accompanied by cirrhosis of the liver. A genetic analysis showed that this is strictly a hereditary disease and that it appears only in so-called homozygous individuals, that is, in those cases in which both parents have corresponding genes. There is extensive damage to the central nervous system with symptoms resembling psychoses. A most unique, purely chemical cause has been discovered for this disease. The patients secrete large quantities of copper with their urine. One could expect that this was the result of an increased copper content in the blood and that the excess had been filtered through the kidneys. As a matter of fact, the opposite turned out to be true: the copper content of the blood had been reduced. The cause turned out to be as follows: the blood contains copper chiefly in the form of a solid compound with protein, but with one specialized protein, the so-called ceruloplasmin. In Wilson's disease there is a sharp decrease in the ceruloplasmin content of the blood plasma. Thus the copper is no longer held properly in the circulatory system and is discharged from

the organism. Moreover, the copper apparently can readily penetrate from the circulatory system into different tissues in increased quantities, which can be accompanied by various disorders. Although the normal amount of copper is essential for the organism, apparently any excess of copper is very undesirable.

Insufficient ceruloplasmin is obviously the result of derangement of the synthesis of this protein. An enticing prospect of treating this severe disease is by introducing a ceruloplasmin preparation into the body as we do in another disease, diabetes (also hereditary to a certain extent), which we treat by introducing a protein hormone - insulin. Of course this may not turn out to be so simple; it will be necessary to use the blood of some animal in order to obtain such a preparation and it must in the first place be active in the environment of the human organism and in the second place not cause any undesirable immunological reactions.

The protein which specifically fixes copper was discovered in the brain. It was called cerebrocupreine. There are as yet no data on the amount of it present in Wilson's disease, but there are indications that important differences were discovered from protein taken from patients in respect to the electrophoretic mobility, as compared with that of protein taken from normal persons. That which was observed would remind one very much of the difference in hemoglobin in sickle-cell anemia. It is very probable that we are dealing with a genuine molecular disease - with a disorder of the structure of the protein molecule. Of particular interest here is the fact that this involves the functions of the most vital tissue - the human brain, and involves the highest of all functions - psychic activities. To some extent new prospects are being opened to chemical approaches to study of psychic phenomena.

The trend which can be characterized as functional biochemistry is the dominant trend in the problems of contemporary biochemistry, in particular, its future efforts and its ultimate purpose. We understand this to be the endeavor to give a biochemical, and ultimately, a chemical interpretation of nature and of the mechanism and essential features of definite physiological functions. This is the fundamental and chief purpose of the biochemistry of the future. We already have examples which bear evidence that this objective is feasible; these examples are still few as yet, but they are quite convincing, for they already involve fundamental, vital, and typical features of the living form.

The problem of the mechanism of the transmission of hereditary properties is before us now as the task of decoding the structure of desoxyribonucleic acid and clarifying the chemical mechanisms of its self-reproduction.

The whole problem of biological movement has been transferred to the molecular level. It was observed in the muscle that the contractile protein myosin itself possesses enzyme properties and is at

the same time an enzyme. Through its enzyme action is released chemical energy from its substrate, adenosinetriphosporic acid, and transforms it into mechanical energy. Studies of the nature of the movement of biological subjects throughout the entire animal world, from the cellular nucleus during mitosis, through flagellate movements in sperms and Trypanosoma, to the work of muscles, and even, to our surprise, to the movement of mimosa leaves - showed the presence of the same mechanism in all these cases. From that moment the entire center of gravity of the study of motor functions of living subjects was shifted as a whole to the sphere of molecular events - the interaction of a high-polymer catalyst, contractile protein, with its low-molecular substrate, the energy carrier.

Two other most important biological functions can be considered as most immediately awaiting their interpretation on a genuinely chemical plane. Both of these are functions connected with the transformation of radiant energy - into chemical energy in one case, and into electrical energy in the other. They are photosynthesis in plants and photoreception, the visual function in animals. They are exceedingly attractive problems, but their solution belongs to the objectives and tasks of future research and I shall limit myself to a brief mention of them as vivid examples of the problems of the biochemistry of tomorrow, functional biochemistry in the most genuine sense of this word, where we await the solution of the great problems posed by biology through chemical analysis.

If we look further ahead, then the most enticing objective is the discovery of the nature of nervous activity in chemical terms, beginning with its most elementary form - the functions of the nerve receptor and conductor, the nerve fiber, and finishing with the integrating function of the brain, the central nervous system.

There is no doubt that in the activities of the nervous system, as is the case of all functions of living forms without exception, the decisive role will in the long run belong to the proteins with their catalytic and enzyme properties, their enormous lability, and their exceptional physical and chemical mobility. However, our information in this respect is most limited. I shall note most briefly one form of participation of proteins in the metabolism of the brain which was brought out in research conducted in our laboratory. This was the exceedingly intense interaction of brain tissue proteins with phosphoric acid. Of all the organic phosphorus compounds, in which the brain tissue is so rich, the protein compounds or the so-called phosphoproteins were found (by use of the isotope method) to be the most rapidly changed, that is, they participated most intensely in the processes of tissue metabolism. The phosphoproteins stand on practically the same level as the nucleotide compounds in respect to rapidity of reaction, in the first instance adenosinetriphosphoric acid, which serves in general to involve mineral phosphate in the cycle of biochemical transformations. Study of the role of phosphoric acid as a factor which

participates in involving the brain tissue proteins in the functional exchange of the nervous system opens one of the paths to clarification of the chemical laws upon which the functions of the nerves and the brain are based.

Up to recent times a monopolistic, or at least a wholly dominant, place in the whole scheme of the chemical mechanisms of the functions of the nervous system was held by one substance - acetylcholine and those enzyme systems which cause its transformation - decomposition and inverse synthesis. This system of chemical agents, enzymes, and their substrates has a main role in the transmission of stimulation and is acted upon by a multitude of medicinal and toxic substances - nerve poisons. The role of this system is undisputed, but steady attention has been turned lately toward another substance which possesses high biological activity. This is the so-called serotonin, a derivative of one of the amino acids, tryptophan. Serotonin is the product of the transformation of tryptophan when it is acted upon by two enzymes (Diagram 9). One oxidizes the benzenoid nucleus of tryptophan, adding an oxy group to it, and the second enzyme decarboxylates the side chain with the formation of an amine. Serotonin is 5-oxy-tryptamine. Serotonin has a sharp effect on the blood vessels, from which it has received its name, but in addition to this, it also has a strong effect on the central nervous system, on the functions of the brain, and this is the thing of interest to us at present. It is this action that we are now inclined to consider the most important, even stating the proposition [24] that serotonin "may turn out to be the vital key to learning the biochemistry of the healthy and the sick mind." Such a very categorical statement must be considered the author's responsibility, but in any event there is much in favor of the point that serotonin plays an important role in the activity of the brain. Confirmation of this can be seen in certain research done on the effect of chemical substances which cause unique derangements in psychic activities, especially those which lead to the occurrence of hallucinations and states which remind one very much of the picture of a severe mental disease - schizophrenia. There is talk already of the possibility of causing "experimental psychoses." The diethylamide of lysergic acid has a specially strong effect of this sort (Diagram 9).

One may regard the presence of a section which has structural outlines closely resembling those of the structure of serotonin as the basis of the structure of this substance, the same indole skeleton, formed by condensation from a five-carbon group with nitrogen, which is alkylized like the nitrogen of the tryptamide part. The basis of the action of the above "hallucinatory" poison is considered to be in the similar structural outlines of the diethylamide of lysergic acid and serotonin, on the principle of action of the so-called antimetabolites. These are substances which replace natural products of metabolism, the metabolites, due to the similarity of their chemical

structure, thereby disrupting the processes of metabolism. When tested on other subjects (this was found to be impracticable in the case of the brain, due to complications created by the low permeability of the brain tissue), it was discovered that the diethylamide of lysergic acid blocked the action of serotonin even in very low concentrations. Serotonin is believed to have a regulatory action in the brain which ensures fine coordination and harmony in the complicated complex of processes which form the basis for psychical activities. Blocking this action of serotonin as a result of disrupting its normal metabolism in brain tissue or under the influence of poisons gives rise to the previously mentioned phenomena of hallucinations and the more severe derangements observed in schizophrenia. On the other hand, serotonin is supposed to participate in the action of tranquillizing substances. In fact reserpine, which is widely used at present, causes significant quantities of serotonin to be released in the brain, and it is believed that it is serotonin which causes the calming of psychical activities actually observed at this time.

There is no need to close our eyes to the fact that the problem of chemical approaches to discovering the principles of the activities of the higher parts of the central nervous system, and ultimately the nature of psychical activities and their disorders, is a field where flights of fancy often far outstrip factual knowledge and contemporary methodological possibilities. Even when we take into account the multitude of limitations, obstacles, and the indirect nature of many conclusions, however, there is no doubt that this problem is one of the most urgent and enticing of all the problems which stand before the chemistry which strives to penetrate into the secrets of biological phenomena. Research is taking its first steps in this field. It is important, however, that the possibilities have been outlined in principle of investigating the phenomena of psychical activities and their disorders in the light of the participation and the influence of chemical substances, and that chemistry is beginning to become involved in this field.

Thus we see the projected indications of what may be regarded, to use A. N. Nesmeyanov's apt expression, as the first breakthroughts by chemistry into the highest stages of biological problems. It is clear that the activities of our central nervous system constitute this highest stage and that its most nighly perfected manifestation is psychical activity.

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FIGURE APPENDIX

СХЕМА 1

Редупликация ДНК

А — аденин; Т — тимин; Г — гуанин; Ц — цитозин

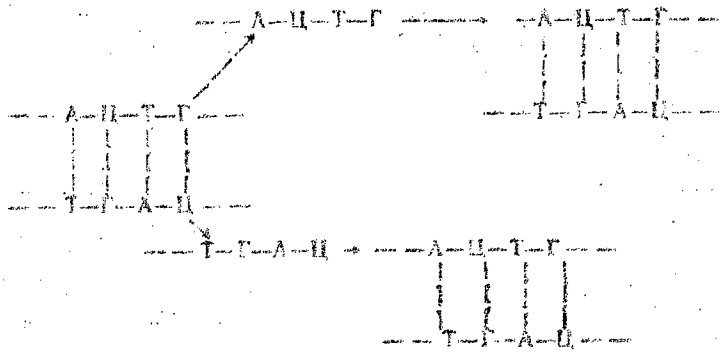


Diagram 1. Reduplication of DNA
A - adonine; T - thymine; G - guanine; C - cytosine

A - C - T - G

A - C - T - G

T - G - A - C

A - C - T - G

T - G - A - C

T - G - A - C

A - C - T - G

T - G - A - C

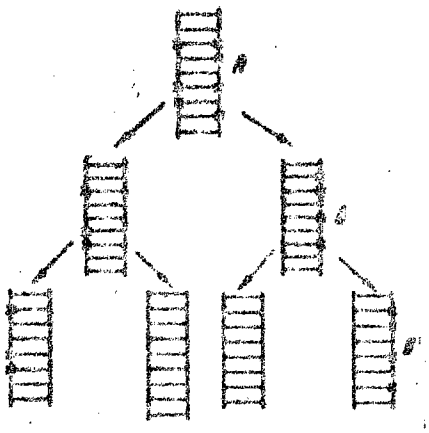


Рис. 1. Распределение изотопной метки в двойных цепях ДНК (крестики — изотопная метка — тритий)
А — родительская ДНК, *Б* — ДНК первого поколения, *В* — ДНК второго поколения

Figure 1. Distribution of the tagged isotope in double DNA chains (crosses represent the tagged isotope tritium).

A - parent DNA; B - first-generation DNA; C - second generation.

A

B

C

СХЕМА 2

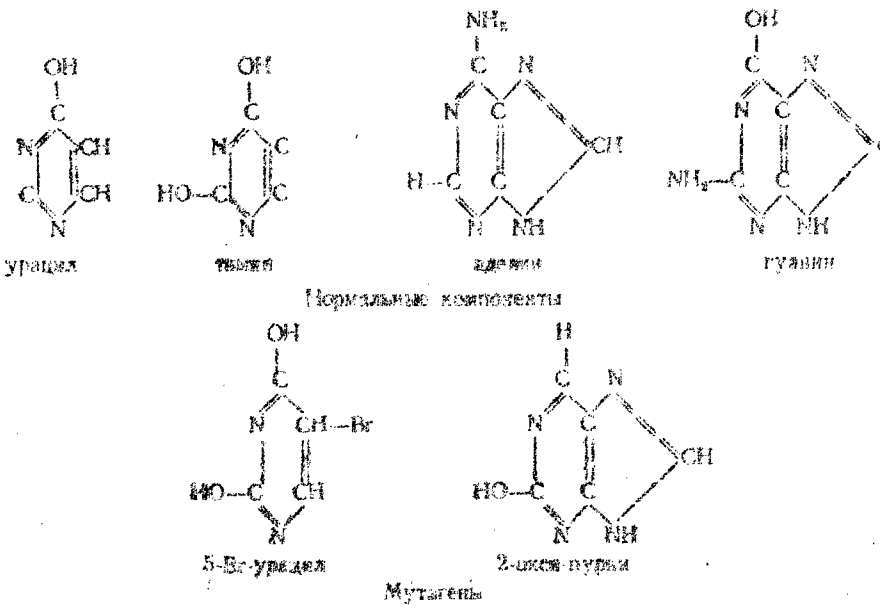


Diagram 2.

uracil thymine adenine guanine

Normal components

5-bromo-uracil 2-oxy-purine

Mutagens

Схема 3

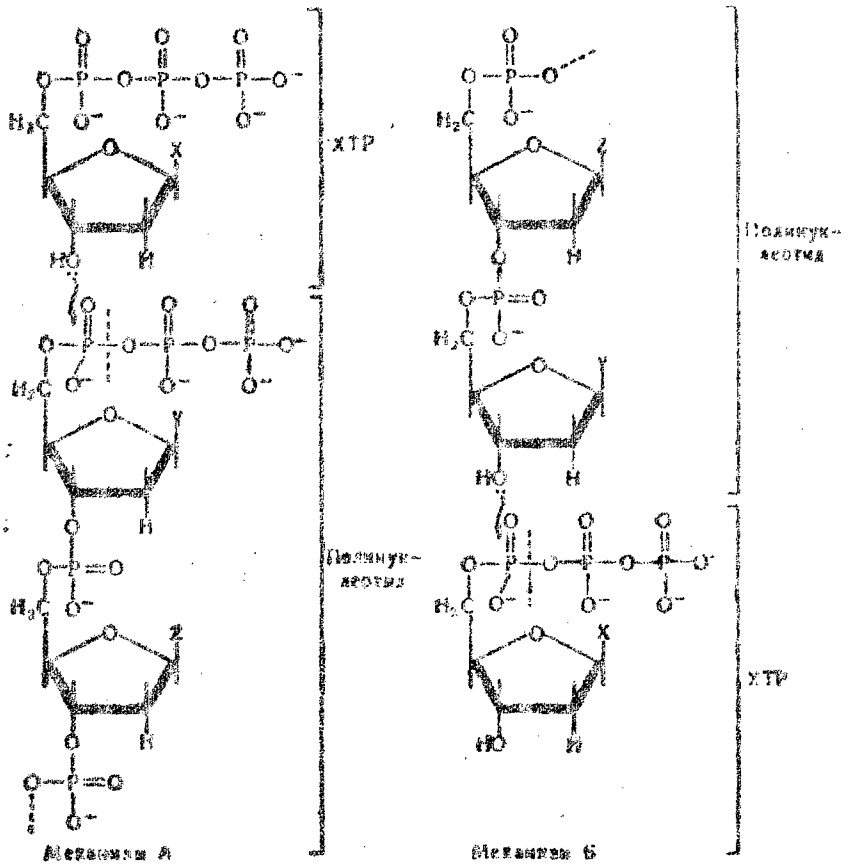


Diagram 3.

Polynucleotide

Polynucleotide

Mechanism A Mechanism B

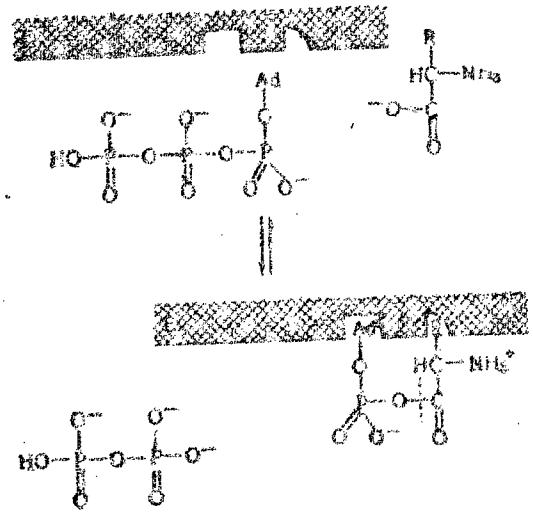


Рис. 2. Активирование аминокислот
E — молекула энзима, Ad — аденозин, R — боковая цепь аминокислоты

Figure 2. Activation of amino acids.

E - enzyme molecule; Ad - adenosine;

R - side chain of the amino acid

Рис. 3. Этапы синтеза белка

Figure 3. Stages in the synthesis of protein.

pH-5 enzyme Soluble RNA Microsome RNA

-ATP -GTP

Free amino acid Protein

Aviation Transportation Ordering, peptidization

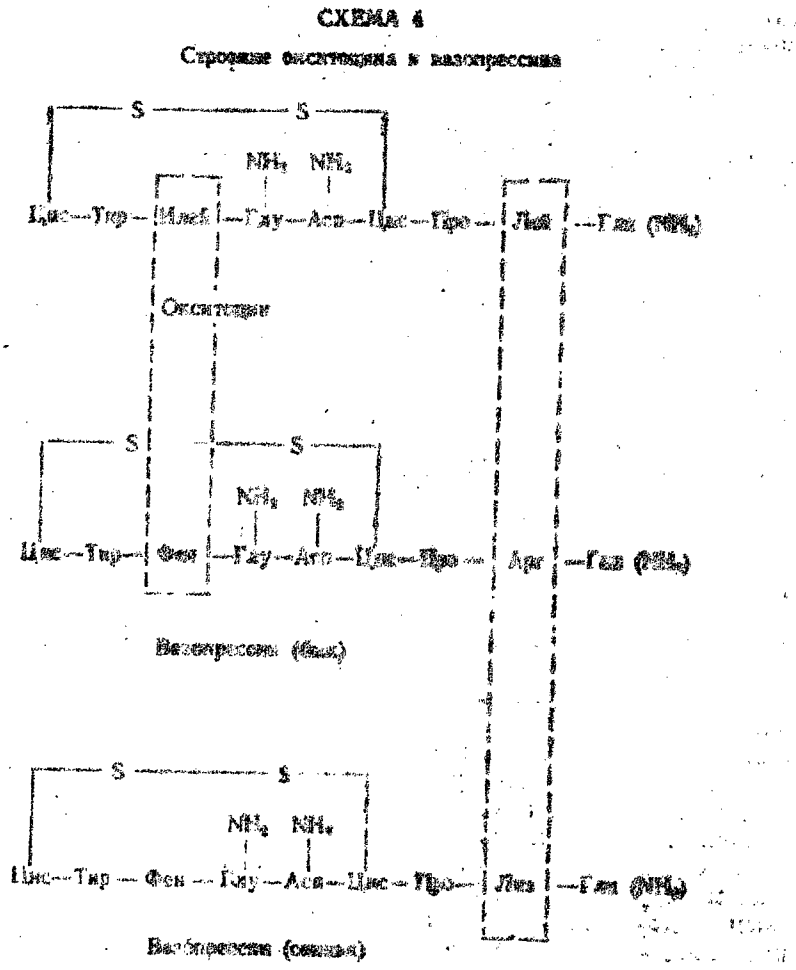


Diagram 4. Structure of Oxytocin and Vasopressin.

Cys - Tyr - Iso leu - Glu - Asp - Cys - Pro - Leu - Gly (NH_2)

Oxytocin

S S

Cys - Tyr - Phen - Glu - Asp - Cys - Pro - Arg - Gly (NH_2

Vasopressin (cattle)

S S

Cys - Tyr - Phen - Glu - Asp - Cys - Pro - Iys - Gly (NH_2)

Vasopressin (pig)

СХЕМА 5

Инсулины

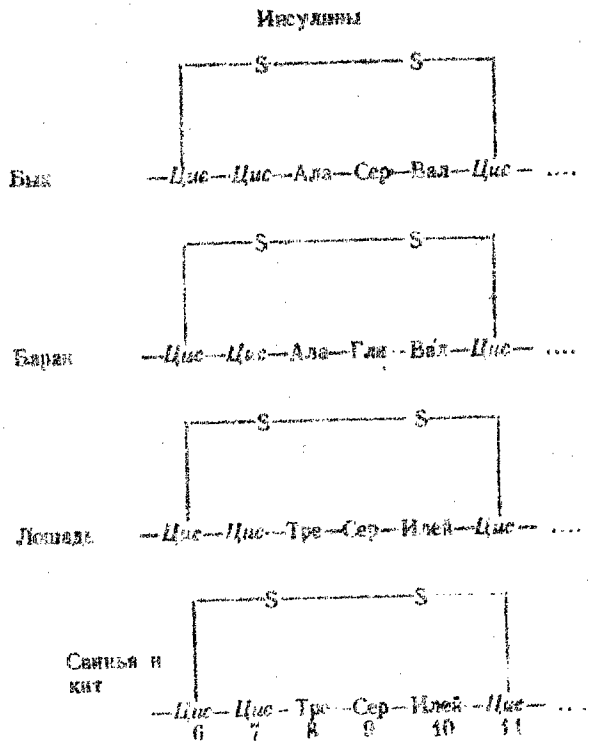


Diagram 5.

Insulins

			S						S			
Cattle	Cys	-	Cys	-	Ala	-	Ser	-	Val	-	Cys	
			S						S			
Sheep	Cys	-	Cys	-	Ala	-	Gly	-	Val	-	Cys	
			S						S			
Horse	Cys	-	Cys	-	Thre	-	Ser	-	Iso leu	-	Cys	
			S						S			
Pig and whale	Cys	-	Cys	-	Thre	-	Ser	-	Iso leu	-	Cys	

СХЕМА 6

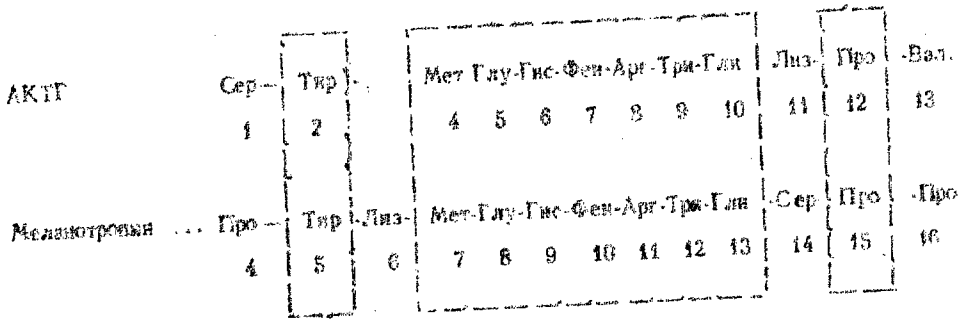


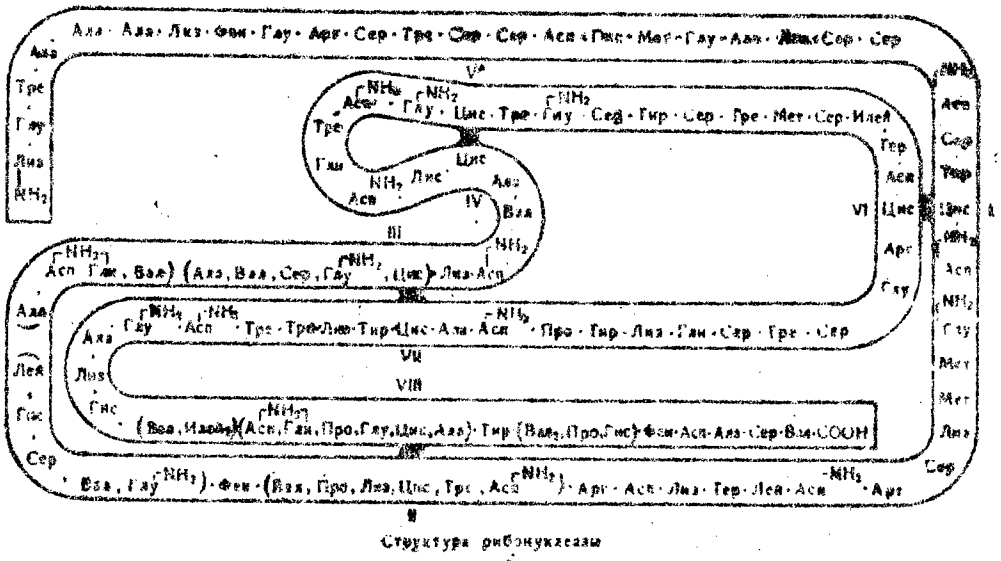
Diagram 6.

ACTH Ser - Tyr Met Glu - His - Phen - Arg - Try - Gly

Lys Pro Val
13

Melano- Pro - Tyr-Lys - Met Glu - His - Phen - Arg - Try - Gly
tropin

Ser Pro Pro
16

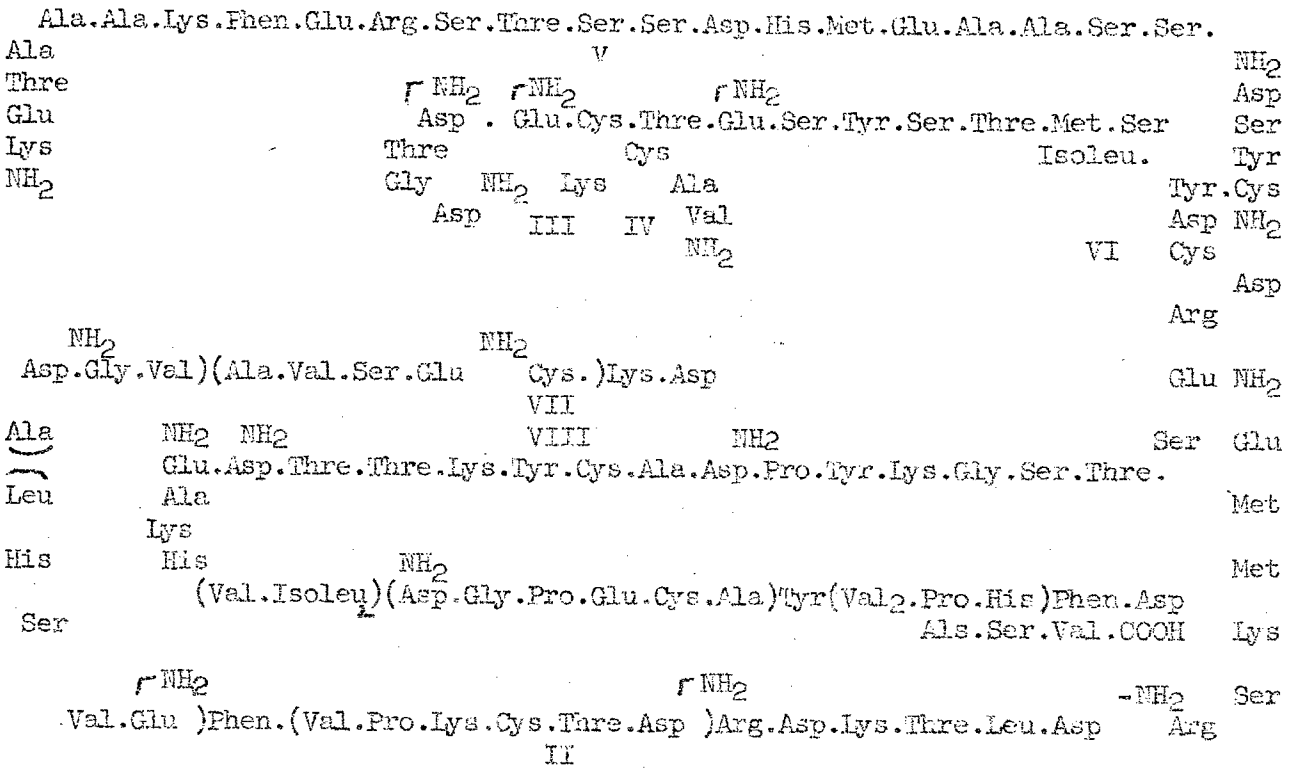


Структура рибонуклеазы

Рис. 4

Figure 4. The structure of ribonuclease.

Legend to Figure 4.



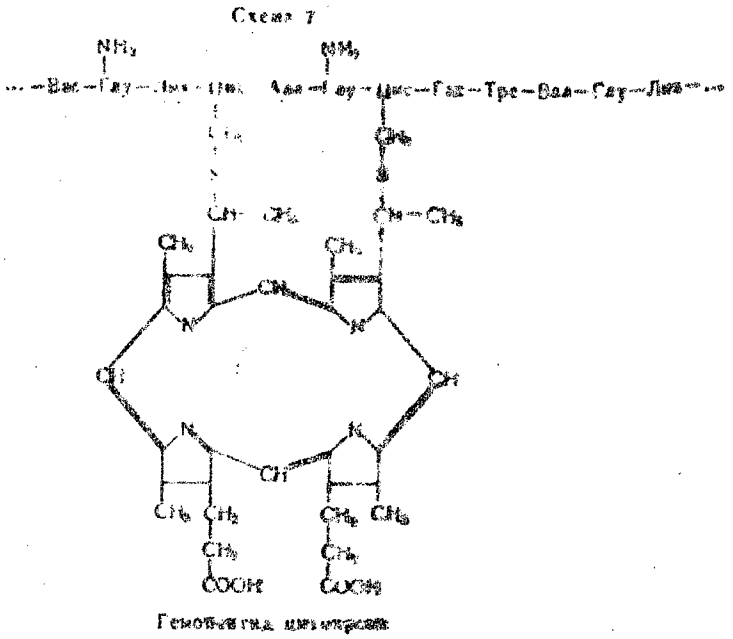


Diagram 7. Hemopeptide of a cytochrome.

NH_2 NH_2

-Val-Glu-Lys-Cys-Ala-Glu-Cys-His-Thre-Val-Glu-Lys-

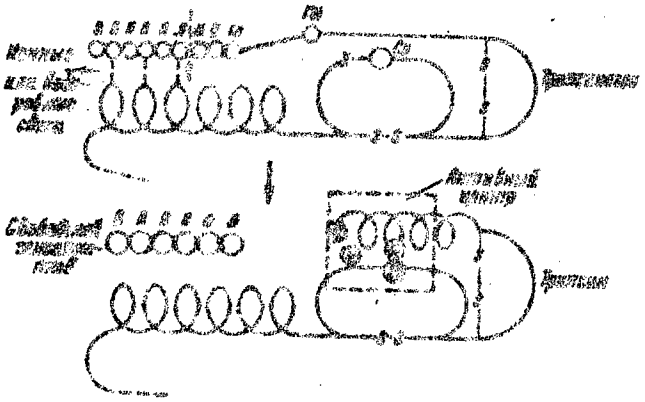


Рис. 5. Активирование трипсиногена
А — аспарагин, *Гл* — глицин, *Ги* и *Н* — гистидин, *И* — изолей-
цин, *Л* — лизин, *Се* — серин, *В* — валин

Figure 5. Activation of trypsinogen.
A - asparagin; Gl - glycine; Gi or H - histidine;
I - isoleucine; L - lysine; Se - serine; V - valine

Gi
V A A A A L I V Gl Se
Ion or Trypsinogen
hydrogen
bonds

V A A A A L Active center
Free V H
I
hexapeptide Se Trypsin

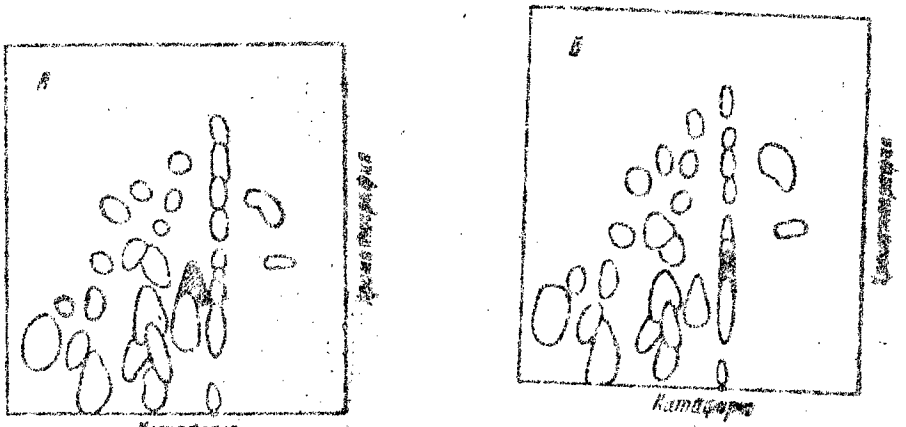


Рис. 6. «Химическая дактилограмма» гемоглобинов.
А — серповидный гемоглобин, Б — нормальный гемоглобин

Figure 6. "Chemical fingerprint" of hemoglobins.
A - sickle-like hemoglobin; B - normal hemoglobin.

A

Chromatography

Cataphoresis

B

Chromatography

Cataphoresis

СХЕМА 8

Модификации гемоглобина

Гб–А (нормальный) .. –Гис–Вал–Лей–Лей–Тре–Про–Глу–Глу–Лиз– ...

Гб–S (серповидный) .. –Гис–Вал–Лей–Лей–Тре–Про–Вал–Глу–Лиз– ...

Гб–С .. –Гис–Вал–Лей–Лей–Тре–Про–Лиз–Глу–Лиз– ...

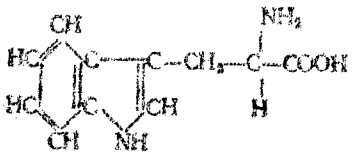
Diagram 8. Modifications of hemoglobin.

Hemoglobin A (Normal) -His-Val-Leu-Leu-Thre-Pro-Glu-Glu-Lys-

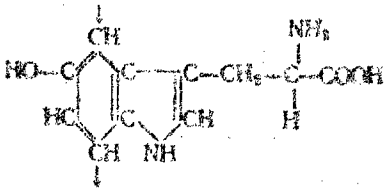
Hemoglobin S (Sickle-shape) -His-Val-Leu-Leu-Thre-Pro-Val-Glu-Lys-

Hemoglobin C -His-Val-Leu-Leu-Thre-Pro-Lys-Glu-Lys-

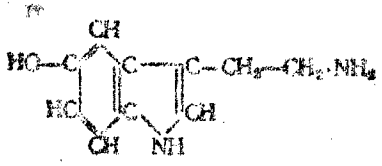
СХЕМА 9



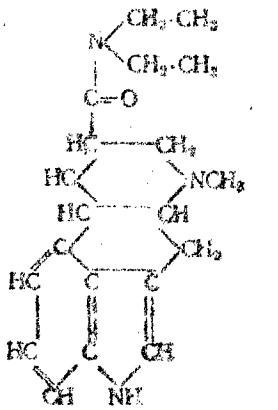
Триптофан



5-окси-триптофан



Серотонин
(5-окси-триптамин)



Диэтил-амид лизергиновой кислоты

Diagram 9.

Tryptophan

5-oxy-tryptophan

Diethyl-amide of lysergic acid

Serotonin
(5-oxy-tryptamine)

5809

- END -